ab65338

Sarcosine Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Sarcosine levels in various samples

This product is for research use only and is not intended for diagnostic use.

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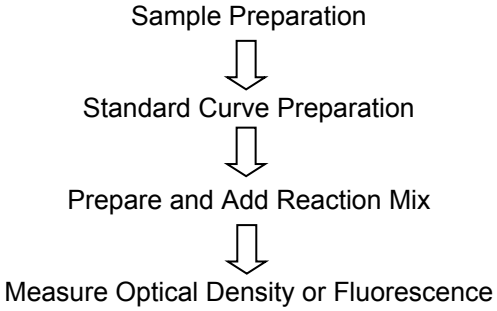
1. Overview

Sarcosine, a natural amino acid, plays important roles as intermediate in the metabolism of choline, methionine, glycine, glutathione, creatine, purine and serine, etc. Detection of sarcosine level has wide applications in research and development.

Abcam's Sarcosine Assay Kit provides an accurate, convenient measure of sarcosine in variety biological samples. In the assay, sarcosine is specifically oxidized to generate a product that converts a colorless probe to a product with intense red color (λ_{max} = 570 nm) and which is also highly fluorescent (Ex/Em = 538/587 nm). Sarcosine is therefore easily detected by either colorimetric or fluorometric methods with detection range 1-10000 µM.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Sarcosine Assay Buffer	25 mL
Sarcosine Probe (in DMSO, anhydrous)	0.2 mL
Sarcosine Enzyme Mix (Lyophilized)	1 vial
Sarcosine Standard (10 µmol; Lyophilized)	1 vial

* Store kit at -20°C.

SARCOSINE ASSAY BUFFER: Ready to use as supplied. It may be stored at +4°C or -20°C.

SARCOSINE ENZYME MIX: Dissolve in 220 µl Sarcosine Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months.

SARCOSINE STANDARD: Reconstitute with 100 µl of dH_2O to generate 100 nmol/µl Sarcosine Standard. Dissolve completely. Store at -20°C, stable for 2 months.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader
- 96-well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

Add 0-50 µl of samples to the wells and bring the volume to 50 µl with Assay buffer.

For unknown samples, we suggest testing several different doses to ensure the readings are in the linear range of the standard curve.

Note:

Urine samples do not work well with the assay due to sample interferences.

2. Standard Curve Preparation:

Mix 10 µl reconstituted Sarcosine Standard with 990 µl of Assay Buffer, mix to generate 1 nmol/µl standard working solution. Add 0, 2, 4, 6, 8, 10 µl of the working solution to 6 consecutive wells. Bring the volume to 50 µl each well Assay Buffer.

Note:

If a more sensitive method is desired, fluorescence can be utilized. Further dilute the standard 10-100 fold, and follow the same procedure as for the colorimetric assay.

3. Reaction Mix: Mix sufficient reagent for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing the following components, mix well:

Sarcosine Assay Buffer	46 µl
Sarcosine Enzyme	2 µl
Probe*	2 µl

Mix well. Add 50 µl of the Reaction Mix to each well containing the Sarcosine Standard or test samples, mix well. Incubate at 37°C for 1 hour.

***Note:**

If the background is high in fluorescence assay, 1/10 probe can be used, which will decrease background significantly.

4. Measurement: Measure 570nm in a microplate reader or fluorescence at Ex/Em = 538/587 nm.

5. Data Analysis

Subtract reagent background from all readings. Plot readings vs. nmoles of Sarcosine. Apply sarcosine readings to the standard curve. Sarcosine concentration:

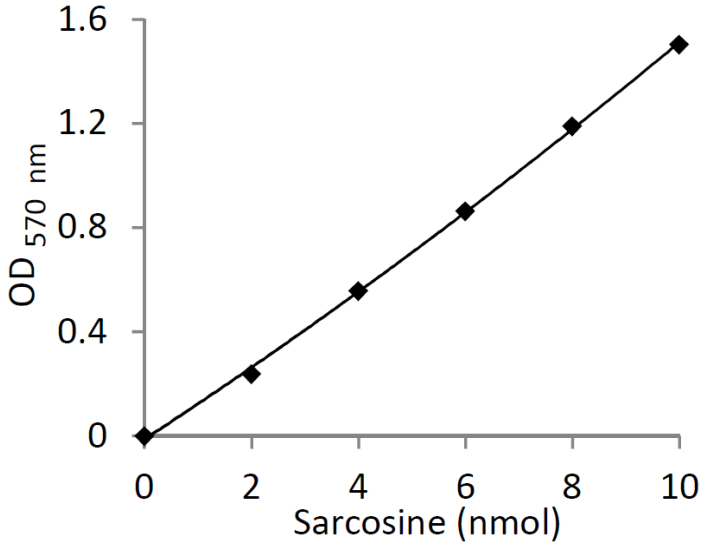
Concentration = Sa / Sv (nmol/µl, or mM)

Where:

Sa is the sample amount of unknown (nmol) from the standard curve.

Sv is the sample volume (µl) added to the reaction well.

Sarcosine molecular weight: 89.10



Sarcosine Standard Curve: Performed following the kit protocol.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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